

Human Neuron Specific Enolase (NSE)

For Quantitative Determination of
Neuron specific enolase In Human Serum

For In Vitro Research Use Only

Human Neuron Specific Enolase (NSE) ELISA KIT

Cat. No. 0050; Kit Contents: (reagents for 96 tests)

Components	96 tests
Streptavidin coated microwell strip plate (96 wells), Cat. # 0051	1 plate
Human NSE Std. A or sample diluent 0 ug/L, 11 ml, Cat. # 052	1 vial
Human NSE Std. B , 0.5 ml 5 ug/L, Cat. # 053	1 Vial
Human NSE Std. C , 0.5 ml 25 ug/L, Cat. # 054	1 Vial
Human NSE Std. D , 50 ug/L 0.5 ml, Cat. # 055	1 Vial
Human NSE Std. E , 100 ug/L, 0.5 ml Cat. # 056	1 Vial
Human NSE Std. F , 200 ug/L 0.5 ml, Cat. # 057	1 Vial
Control serum low & high (exact values printed on vials) All Stds and Controls MUST BE STORED FROZEN IN Aliquots upon receipt and used once after thawed.	
Biotinylated anti-NSE capture antibody 11 ml Cat. # 058	1 bottle
Anti-NSE-HRP Conjugate , 11 ml, Cat. # 059	1 bottle
Wash buffer (100X), 10 ml, Cat#W-100; dilute 1:100 with distilled water,	1 bottle
HRP substrate Solution , Cat. #TMB0050, 11 ml	1 bottle
Stop solution, 10 ml, Cat. # T-10	1 bottle
Instruction Manual, M - 0 5 0	1

The glycolytic enzyme enolase (2-phospho-Dglycerate hydrolyase) exists as several dimeric isoenzymes (aa, ab, ag, bb and gg) composed of three distinct subunits: a, b, and g (alpha, beta, and gamma). Three isoenzymes are found in human brain: aa, ag, and gg. The ag and gg-enolase isoenzymes are also known as neuron-specific enolase (NSE) as these isoenzymes initially were detected in neurons and neuronendocrine cells. Lung cancer is one of the most spread cancer forms with incidences about 50-100 per 100,000 population. Approximately 20% of the lung cancer is small cell lung cancer. NSE has been shown to be a valuable tumor marker of neuroendocrine origin, particularly in small cell lung cancer and in neuroblastoma. Patients with small cell lung cancer show various proportions of ag and gg isoenzymes. The determination of NSE should detect ag and gg isoforms with the same sensitivity. The antibodies for this particular assay are specific for the g-subunit without cross reactivity with a or b subunits. NSE is reported to be useful diagnostic marker for lung cancer, neuroblastoma, melanoma, seminoma and in injury of central nervous system. In addition to the above, NSE can be a valuable tool in following-up the effect of chemotherapy of small cell lung cancer, in prognostic evaluation of patients with small cell lung cancer, and in differential diagnosis between cell lung cancer and non-small cell lung cancer.

Various immunoassays, including radioimmuno-assay (RIA), turbidimetry, Immunofluorometric assay, nephelometry and radial immunodiffusion were developed to measure NSE in serum. ADI's NSE ELISA kit provides a sensitive assay for NSE in serum.

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PERFORMANCE CHARACTERISTICS

DETECTION LIMIT - Based on sixteen replicates determinations of the zero standard, the minimum concentration of human NSE detected using this assay is ~ 1.0 ug/L. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Intra-assay precision:

Three pooled sera were assayed 8-times in a single run (CV 4-5%). The inter-assay determinations were performed in duplicate over a period of 1 week.

Serum samples	Mean (ug/L)	Intra-assay		Mean (ug/L)	Inter-Assay	
		S.D.	CV%		S.D.	CV%
A	54.4	3.05	5.61	53.8	6.29	11.65
B	112.7	6.13	5.43	116.8	8.23	7.06
C	213.5	10.4	4.87	226.4	23.4	10.3

LINEARITY

Samples with known concn were spiked with known conc (diluted 1:2 v/v) and NSE values determined.

Initial NSE (ug/L)	NSE Spiked (ug/L)	Expected (ug/L)	Observed (ug/L)	Recovery (%)
5	25	15	14.3	95.3
5	85	45	45.8	101.8
5	158	81.5	84.7	103.9
25	85	55	56.5	102.7
25	158	91.5	97.3	106.3
100	158	129	117.3	90.9

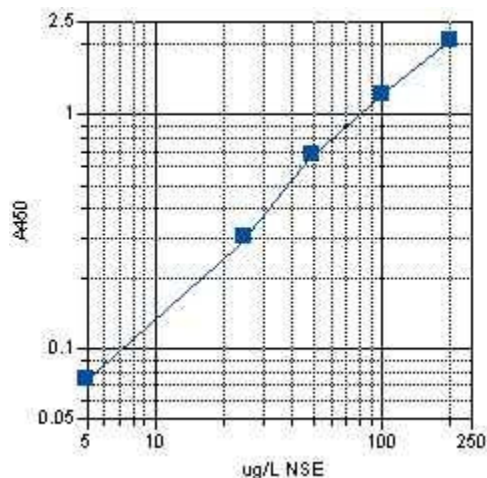
A serum sample with different concentration of NSE was diluted with series of NSE-free serum. The dilutions were tested for NSE.

Sample Dilution	NSE Expected (ug/L)	NSE Observed (ug/L)	Recovery (%)
Undiluted	333		
1:1/5	266.5	287	107.7
1:1/4	249.8	244.4	97.8
1:1/3	222	203	91.4
1:1	166.5	165.5	99.3
1:2	111	112.2	101.1
1:4	83	86.3	104.0

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450nm}	Calculated Conc. (ug/L)
A1, A2	Std. A (0 ug/L)	0.002	
B1, B2	Std. B (5.0 ug/L)	0.080	
C1, C2	Std. C (25.0 ug/L)	0.277	
D1, D2	Std. D (50 ug/L)	0.683	
E1, E2	Std. E (100 ug/L)	1.226	
F1, F2	Std. F (200 ug/L /l)	2.084	
G1, G2	Sample 1	1.087	94.2

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Human Neuron specific enolase (NSE) ELISA kit is based on simultaneous binding of human NSE from samples to two antibodies, a biotinylated capture antibody and HRP-conjugated antibody. Streptavidin coated on the plate then captures the biotinylated antibody-NSE complex. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of NSE present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of NSE in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The NSE ELISA test is intended for *in vitro* research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of reagents

Wash buffer is supplied as 100x stock. **Dilute 10 ml into 990 ml de-ionized or distilled water**, mix, and store at room temp for 1-2 weeks. It can be stored at 4°C for long term storage.

All other reagents are supplied ready to use.

Standards and Control Must be Stored Frozen

NSE standards and controls are unstable at prolonged room temp or 4°C. We recommend that the controls and standards be aliquoted upon receipt in 60 ul aliquots (need 25+25 ul or 50 ul per run in duplicate) and stored at -20°C or below. Thaw the standards/control once before the assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. The **standards & controls must be stored frozen**. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (*ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE*). **Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute wash buffer 1:100 with water. **Dispense 200-300 ul of wash buffer** to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate. Do not dilute standards.
2. Pipet **25 ul of standards**, and available diluted control (no supplied by ADI), and serum samples into appropriate wells in *duplicate*. Immediately dispense 100 ul of biotinylated capture antibody (blue solution). Gently mix the samples, cover the plate and incubate at **room temp for 60 min**.
3. Wash the plate **5X** with wash buffer (300 ul/wash). We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing
4. Add **100 ul antibody-enzyme conjugate** into each well. Mix gently. Cover the plate and incubate for **60 minutes** at room temperature
5. Wash the plate **5X** with wash buffer (300 ul/wash).
6. Dispense **100 ul TMB substrate per well**. Mix gently.
7. Cover the plate and **incubate for 30 min at room temp**. Blue color develops into standard and all positive wells
8. Stop the reaction by adding **50 ul of stop solution** to all wells. Mix gently. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES-

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve standard graph paper by plotting net absorbance values of standards against appropriate protein concentrations. Read off the NSE concentrations of the control and patient samples. Sample values must be multiplied by the dilution factor if samples were diluted (samples >200 ug/L).

Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples. It is possible to use the plasma and other biological fluids. However, the sample volume or dilutions may be adjusted according to the expected concentrations or unknown samples. Crossreactivity with NSE from other species has not been determined.

EXPECTED VALUES

It is recommended that each laboratory determine its own normal and abnormal range. A clinical study of test was conducted for 200 apparently normal serum showed that 95% of individuals have NSE values below 15 ug/L.

HIGH DOSE HOOK EFFECT

No hook effect was observed with samples up to 1500 ug/L.

SPECIFICITY

The antibodies used in this kit are specific for gamma-subunit of NSE (γ -NSE). The antibodies do not detect NSE with other subunits.

Species Crossreactivity

The kit is designed for human NSE. Antibody crossreactivity or the use of this kit in other species (mouse, rat, etc) has not been studied.

References: Paus E and Nustad K (1989) Clin Chem 35, 2034; Paus E and Risberg T (1989) Tumor Biol. 10, 23-30; Cooper EH (1987) Br. J. Cancer 56, 65; Wibe E (1990) Cancer Lett. 52, 29-31; Fossa SD (1992) Br. J. Cancer 65, 297